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Sesquiterpene and long chain ester from *Tanacetum longifolium*☆

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Abstract

A new naturally occurring linear sesquiterpene (tanacetene) (**1**) having irregular non-head- to- tail attachment of isoprene units, a new long chain ester (**2**) and 10 known compounds have been isolated from hexane and chloroform extracts of the roots and aerial parts of *Tanacetum longifolium* wall. The structure of (tanacetene) (**1**) was established as (2*E*,6*E*,10*E*)-2,6,11-trimethyl-dodeca-2,6,10-triene and that of (**2**) as heptatriacontanyl eicosanoate by spectroscopic methods along with 10 known compounds. From the oily fraction twelve volatile compounds were identified by GC–MS. The roots of this plant were found to be a new major source of *Z*-spiroketalenol ether-6,7-epoxy-diyne in 3.2% yield on dry weight basis.

Keywords: *Tanacetum longifolium*; Asteraceae; Sesquiterpene; Tanacetene; Heptatriacontanyl eicosanoate

1. Introduction

Tanacetum longifolium (Asteraceae) is an annual hairy herb growing wild at an altitude of 3000–4500 m from Kashmir to Uttranchal in India (Anon, 1962). The genus is found to contain acetylenes (Bohlmann et al., 1973) and sesquiterpene lactones (Oksuz, 1990). The latter are reported to have antibacterial and cytotoxic activities (Goren et al., 1991) and are main secondary metabolites along with certain flavonoids (Williams et al., 1999). In continuation to our earlier work on volatile composition from the roots and aerial parts (Kaul et al., 1993), a detailed investigation of this species was undertaken by us. Hexane and chloroform extracts of the aerial parts and roots were investigated in detail.

2. Results and discussion

From the hexane extract of the aerial parts we isolated eight known compounds identified as *z*-spiroketalenol ether-6,7-epoxy-diyne (Birnecker et al., 1988), *z*-spiroketalenol-ether-diyne (Bohlmann et al., 1973), β-sitosterol, 1-eicosanol, 1-tetracosanol, 1-docosanol and two sesquiterpene lactones ludartin (Sosa et al., 1989) and artiglasin-A (Lee et al., 1971). From the hexane extract of the roots *z*-spiroketalenol ether-6,7-epoxy-diyne and 1-octadecanol were identified. $CHCl_3$ extract of roots resulted in isolation of an essential oil fraction which on examination by GC was found to contain 21 compounds. Out of these 12 were identified by GCMS (Jenning and Shibamoto, 1980; Adams, 1989) as *cis*-sabinene hydrate (1.7%) *n*-octane (2.6%), *trans*-sabinene hydrate (1.7%), terpin-4-ol (8.9%), *p*-cymen-9-ol (1.8%), *cis*-α-bergamotene (4.2%) cyperene (1.1%) α-himachalene (1.4%) epizonarene (14.7%), *cis*-nerolidol (6.2%), neryl acetone (4.1) and geranyl acetone (2.4%). From the same extract **1** was also isolated. From $CHCl_3$ extract of aerial parts **2** along with 1-octacosanol and a flavonoid jacidin (Roitman and Lynn, 1985) were isolated. The structures of these compounds were established by extensive spectroscopic studies (IR, ^{1}H NMR, ^{13}C NMR, MS) and comparing the data of known compounds with their literature data.

Compound **1** obtained from the $CHCl_3$ extract of the roots has a molecular formula of $C_{15}H_{26}$ (EIMS, M^+ at *m*/*z* 206). It was isolated as an optically inactive oil. Its ^{1}H NMR showed signals at δ 1.60 (s, 9H, 3×CH_3) and δ 1.67 (s, 6H, 2×CH_3) for methyl groups attached to olefinic double bond, two overlapping multiplets at δ 2.0, and 2.06 for two CH_2 each and a broad triplet centered

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at δ 5.11 for three olefinic protons. The mass and ^{1}H-NMR assignments are similar to the reported values of a synthesized compound (Hoppmann and Weyerstahl, 1978). ^{13}C NMR from C-1 to C-8 of **1** resembled the reported values of farnesene (Rahman and Ahmed, 1992) (Table 1). E-configuration of three double bonds at C-2, C-6 and C-10 were evident by the upfield shift of 13,14 and 15 methyls in its ^{13}C-NMR at δ 16.01, 17.65 and 16.01 respectively due to gauche interaction while 1 and 12 methyls did not experience such interaction and appear down field at δ 25.66 (Grazia et al., 1982). The identical chemical shift of Me-13, Me-15 and Me-1, Me-12 showed that the terminal methyls have identical environment. The fusion of one isoprene unit to the second unit is tail-to-tail which is unique as was obvious by attachment of 15-Me at C-11 while this is found at C-10 in head to tail attachment. The tail-to-tail attachment is further supported by the chemical shift of C-9 at δ 28.15 which shows that it is adjacent to an olefinic CH at C-10 and not to a methyl group at this position where it is observed between 39 and 40 ppm as found for C-5 at δ 39.72. The assignments were confirmed by DEPT, HMQC and HMBC (Fig. 1) experiments. On the basis of above assignments the structure of this sesquiterpene hydrocarbon was established as (2*E*,6*E*,10*E*)-2,6,11-trimethyl-dodeca-2,6,10-triene and was designated as tanacetene. It is the first report of this type of tail-to-tail fusion of two isoprene units at C-9 position in the sesquiterpene class of compounds and reported by us for the first time from natural source.

Table 1
^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) of **1** in $CDCl_3$[a]

Position	^{1}H	^{13}C
1	1.60 (*s*)	25.66 q
2	–	135.08 *s*
3	5.18 (*t*)	124.27 *d*
4	2.06 (*m*)	26.75 *t*
5	2.00 (*m*)	39.72 *t*
6	5.18 (*t*)	131.22 *s*
7	–	124.39 *t*
8	2.06 (*m*)	26.65 *t*
9	2.00 (*m*)	28.15 *t*
10	5.18 (*t*)	124.27 *d*
11	–	134.87 *s*
12	1.60 (*s*)	25.66 *q*
13	1.67 (*s*)	16.01 *q*
14	1.67 (*s*)	17.65 *q*
15	1.67 (*s*)	16.01 *q*

[a] Assignments confirmed by DEPT and HMQC experiments.

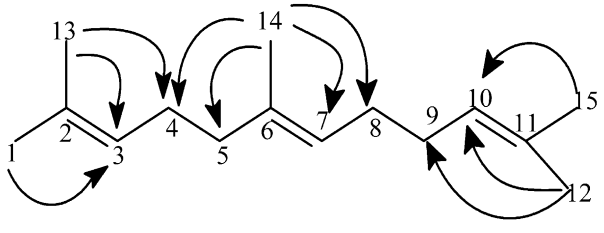

Fig. 1. Proton–carbon long range correlation in HMBC spectrum of **1**.

The irregular non-head-to-tail terpenes are an interesting class of natural products as they do not follow the biogenetic isoprene rule and are of rare occurrence. Although, some cyclic sesquiterpenes are reported from *Thapsia villosa* of apiaceae family, these reports include thapsan and thapsane derivatives like (8*R*, 14*S*)-8-angeloyloxy-thapsan-14-ol (Lemmich et al., 1984), derivatives of 14,15-epoxy-thapsan-14-ol (Pascual Teresa et al., 1986a), 15-acetoxy thapsan-14-al, (15)-1-senecioyloxy-6(14)-thapsan-15-ol and related compounds (Pascual Teresa et al., 1986b) and three hydroindene sesquiterpenes having thapsane skeletons (Smitt et al., 1990). Some irregular cyclic diterpenes have also been reported and the probable pathway leading to biosynthesis of some irregular sesquiterpenes and diterpenes has been discussed (van Klink et al., 2000) which most probably are formed by head-to-head condensation of a monoterpene and a hemiterpene unit. The irregular monoterpenes having non-head-to-tail linkage seem to be mostly prevalent to the members of Asteraceae family. Their biogenesis in plant is related to the formation of steroid precursor squalene (Epstein and Gaudioso, 1984; Dewick, 1998) and their probable mechanism is well discussed (Dewick, 1998). *T. longifolium* also belongs to the Asteraceae and isolation of non-head-to-tail linear sesquiterpene tanacetene assumed added interest for its possible biogenesis in plant. We propose the most probable pathway for its biogenesis (Fig. 2) that it may be formed by condensation of a geranyl diphosphate (GPP) and dimethylallyl diphosphate (DMAPP) (instead of isopentenyl diphosphate (IPP) as found in regular sesquiterpenes). The possible biosynthetic pathway is probably the same as that proposed in the formation of squalene (Dewick, 1998).

The compound **2** obtained as colourless solid from $CHCl_3$ extract of the aerial parts was analyzed for $C_{57}H_{114}O_2$ (M^+ m/z 830). Its IR spectrum showed absorption at 1730 and 1265 cm^{-1} for ester, 2910, 2842, 1380, 725 and 715 cm^{-1} for long chain. In its ^{1}H NMR spectrum a triplet at δ 4.05 (J = 6.5 Hz) and a signal at 64.35 ppm in ^{13}C NMR were assigned to oxymethylene moiety. The two methylene protons adjacent to keto group appeared at δ 2.28 as triplet (J = 6.5 Hz) in ^{1}H NMR and the carbon signal of the same methylene group appeared at δ 34.40 in ^{13}C NMR. A carbon signal of double intensity at δ 31.89 was assigned to methylenes, one adjacent to oxymethylene and the other adjacent to the ketomethylene. Protons corresponding to these methylenes were observed at δ 1.64 as a multiplet. The signal of carbonyl carbon was observed at δ 173.94. Six protons of terminal methyl groups appeared as broad triplet at δ 0.88 and the corresponding carbons appeared at δ 14.06 in its ^{13}C NMR. It was ascertained

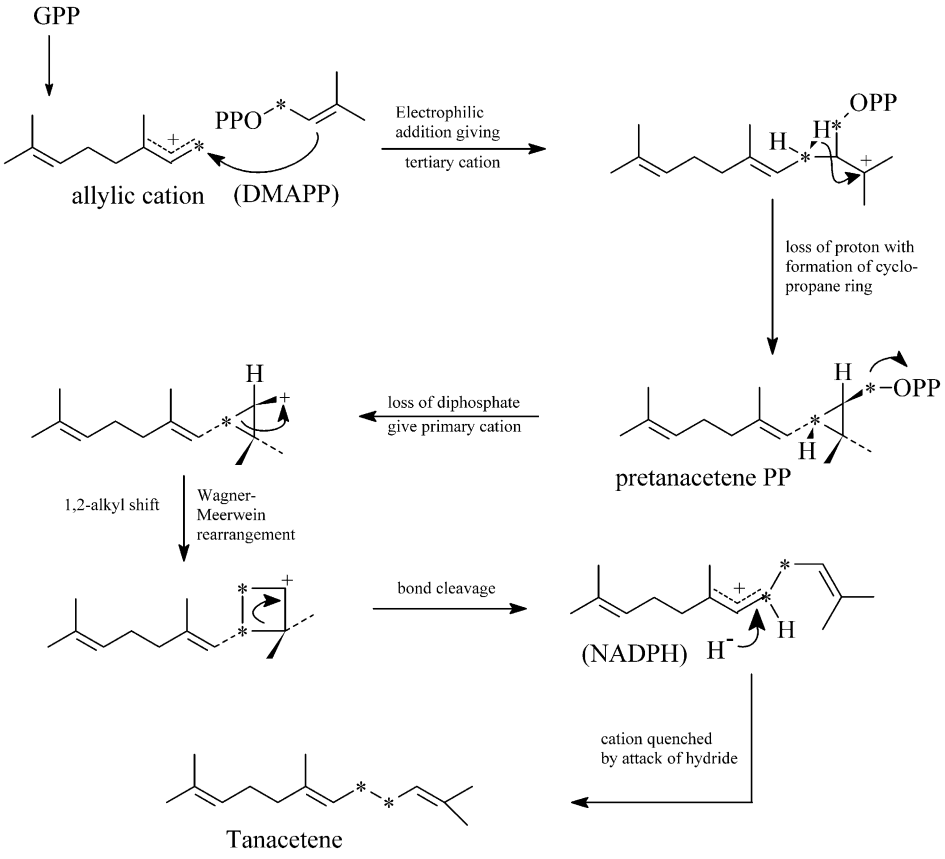

Fig. 2. Proposed pathway for biosynthesis of tanacetene.

from DEPT experiment that no tertiary carbon (except C=O) and methine (CH) carbon were present, so the possibility of any branching was ruled out. From the above discussion it was clear that it is an ester of a linear long chain alcohol and a long chain acid. It contains 114 protons as calculated from ^{1}H NMR and also confirmed the molecular formula. The acid moiety was confirmed by its MS fragmentation pattern (Fig. 3) which was typical of long chain compounds as shown by gradual decreasing the intensity of mass fragments but an intense peak at *m*/*z* 313 (a) showed the cleavage of the alkyl moiety by transfer of 2H to the acid moiety which is characteristic of long chain esters and a prominent peak at *m*/*z* 295 of (b) (Silverstein et al., 1981) confirming the acid moiety as $C_{20}H_{39}O_2$ and leaving the alkyl moiety as $C_{37}H_{75}$. The above data confirmed the structure as heptatriacontanyl-eicosanoate **2**.

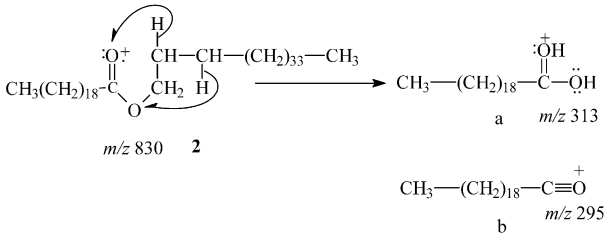

Fig. 3. Mass spectrometric cleavage of **2**.

3. Experimental

3.1. General

Melting points were recorded on a Mettler FP800 (Central processor) and are uncorrected. IR spectra were measured on a Jasco FT/IR-5300, 1D and 2D NMR were recorded in $CDCl_3$ on a Bruker DRX-300 MHz (^{1}H) and 75 MHz (^{13}C) instrument using TMS as internal standard. EIMS were recorded on a JEOL JMS HX-100 mass spectrometer at 70 ev. GC was carried out using a Shimadzu GC-14B carbowax 20M, capillary column, length 30 m, ID = 0.25, FID, Nitrogen as carrier gas, temp programme and GCMS on Perkin-Elmer Q-Mass 910 mass spectrometer fitted with carbowax 20M capillary column, other parameters same as in GC. C.C. was carried out on silica gel-G (60–120 mesh) and TLC on silica gel (Merck).

3.2. Plant material

The aerial parts and roots of *T. longifolium* wall. were collected from Dhauladhar hills at a height of 3000 m in Himachal Pradesh, India, located at 32^0 6′ N, 76^0 18′ E. An authenticated voucher specimen (No. 298 BKS) has been deposited in the herbarium of our institute.

3.3. Extraction and isolation

1 kg of each air dried roots and aerial parts of the plant were extracted with *n*-hexane (2.5 l×4) which yielded 90 and 55 g of the extract respectively. Subsequent extraction of roots with $CHCl_3$ (2.5×4) and of aerial parts *n*-hexane (2.5 l×4) yielded 32 and 21 g of the extracts, respectively.

50 g hexane extract of the roots was subjected to normal phase silica-gel column chromatography (7.5×120 cm column) and eluted with C_6H_{14}, C_6H_{14}: EtOAc and EtOAc gradient followed by EtOAc:MeOH (90:10). From hexane:EtOAc (98:2), the major compound *z*-spiroketalenol ether-6,7-epoxy-diyne (17.5 g) was obtained in pure form in 3.2% (dry wt basis). Subsequent fractions gave 1-hexadecanol. $CHCl_3$ extract (10 g) of roots by normal phase column chromatography (7.5×70 cm) afforded a fraction of essential oil (95 mg) which was examined by GC and GC–MS (see Discussion) and found to contain 21 compounds, 12 of them were identified. The second fraction was also an oil found to be a single compound (**1**). The purity of this compound was checked by GC.

The *n*-hexane extract (50 g) of aerial parts on normal CC yielded eight compounds. From *n*-hexane:EtOAc (98:2), *z*-spiroketalenol ether-6,7-epoxy-diyne (25 mg); *n*-hexane: EtOAc (95:5) yielded a mixture of two compounds which were separated by repeated C.C. to obtain 1-eicosanol and β-sitosterol; from *n*-hexane:EtoAc (95:10) tetracosanol (122 mg) was isolated. Ludartin (210 mg) and artiglasin-A, (105 mg) were obtained from *n*-hexane:EtOAc (80:20), 1-docosanol (456 mg) and subsequent fraction of same eluting solvent yielded *z*-spiroketalenol ether diyne. The $CHCl_3$ extract of aerial parts afforded three compounds, compound **2** eluted in *n*-hexane:EtOAc (95:5) and subsequent fraction afforded octacosanol while from EtOAc: MeOH (99:1) a flavonoid jaicedin (512 mg) was obtained.

3.4. 2,6,11-Trimethyl-dodeca- 2,6,10- triene (tanacetene) (*1*)

Light yellow oil, 20 mg, ^{1}H NMR, ^{13}C NMR and 2D NMR (see Table 1), MS (*m*/*z*) M^+ 206 (5) 164 (18) 137 (9) 123 (15) 95 (22) 82 (65) 69 (100) (Found C 87.16, H 12.84, $C_{15}H_{26}$ *require* C 87.38, H 12.62).

3.5. Heptatriacontanyl eicosanoate (*2*)

MP 73.9 °C (15 mg), IR, υ_{max}^{KBr} 2910, 2842, 1730, 1460, 1380, 1265, 725, 715 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (6H, t, J=6.5 Hz, 2×CH_3) 1.25 (100H br s, 50×CH_2) 1.64 (4H, m, 2×CH_2) 2.28 (2H, t, J=6.5, CH_2) 4.05 (2H, t, J=6.5, CH_2) ^{13}C NMR (75 MHz $CDCl_3$, DEPT) 14.06 (terminal 2×CH_3) 22.62 (2×CH_2) 25.01 (CH_2) 25.91 (CH_2) 28.64 (CH_2) 29.24–29.66 (45×CH_2) 31.89 (2×CH_2) 34.40 (CH_2) 64.35 (CH_2) 173.94 (C=O) EIMS (C_{57} H_{114} O_2) *m*/*z* (rel. int.) 830(0.1) 733(0.3) 705(0.5) 621(1) 593(1) 519(1.2) 437(0.6) 313(15) 311(2) 295(5) 111(20) 97(40) 83(40.5) 71(50) 57(100) 43(52) (found C 80.21, H 13.95. $C_{57}H_{114}O_2$ require: C. 80.96 H 13.73).

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